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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : A23G 9/02 | A1

(11) International Publication Number: WO 98/04146

(43) International Publication Date: 5 February 1998 (05.02.98)

(21) International Application Number: PCT/EP97/03635

(22) International Filing Date: 4 July 1997 (04.07.97)

(30) Priority Data:
96305497.8 26 July 1996 (26.07.96) EP
(34) Countries for which the regional or international application was filed: AT et al.
96305498.6 26 July 1996 (26.07.96) EP
(34) Countries for which the regional or international application was filed: AT et al.

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FROZEN FOOD WITH ANTIFREEZE PEPTIDES

(57) Abstract

A process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.

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FROZEN FOOD WITH ANTIFREEZE PEPTIDES

Technical Field of the Invention

The invention relates to a process for the preparation of a food product containing AFPs and to food products containing AFPs.

Background to the Invention

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs.

Antifreeze proteins have been described in the literature, see for example Marilyn Griffith and K. Vanya Ewart in Biotechnology Advances, Vol 13, No 3, pp 375-402, 1995. Antifreeze properties generally possess one or more of the following properties: thermal hysteresis, inhibition of ice recrystallisation, control of ice crystal shape and interaction with ice nucleators.

Thermal hysteresis is the best known property of AFP's and the property is normally used to test for the presence of AFP's. Thermal hysteresis results from a lowering of the apparent freezing temperature of a solution containing a thermal hysteresis active AFP without affecting the melting temperature. The identification of sources of AFP by thermal hysteresis tests is widely described in the literature, see for example John G Duman in Cryobiology 30, 322-328 (1993).

Inhibition of ice recrystallisation is another property of AFPs. This activity is also referred to as ice crystal growth suppression. This property can be tested by comparing at a certain point in time the ice crystal size of crystals in the presence of AFP and in the absence of AFP. The application of

this method in the testing of fish AFPs is described in US patent 5,118,792 (DNA Plant Technology Corporation)

A third property of AFPs is their ability to influence the shape of ice crystals. This property stems from the selective binding of AFPs to certain faces of the ice crystal and therewith limiting crystal growth in certain directions. The presence of ice crystals having an hexagonal bipyramid shape is then considered indicative of the presence of AFP. This method is for example described for testing the activity of extracellular winter rye AFPs in WO 92/22581 (University of Waterloo).

A fourth property of AFPs is their ability to inhibit the activity of ice nucleating substances. This interaction between and AFP and an ice nucleator may for example result in increased thermal hysteresis. This property is for example tested in WO 96/40973 (University of Notre dame du Lac)

AFPs have been suggested for improving the freezing tolerance of products. Many applications have been suggested in this context.

For example AFPs have been suggested for enhancing the cryopreservation of biological materials (WO 91/12718, Agouron Pharmaceuticals, WO 91/10361, The Regents of the University of California). Also AFPs have been suggested to prevent leakage from liposomes e.g. in cosmetic or pharmaceuticals (see WO 96/20695). A further possible application is to increase the freezing tolerance of plants by including therein (or transgenetically producing therein) an AFP (See J. Cell. Biochem. Suppl. vol. 14e, 1990, page 303 XP002030248, Lee et al, abstract R228). Also fish AFPs have been suggested for use in food products for example in frozen yoghurt or ice cream (US 5,620,732 Pillsbury and WO 96/11586, HSC Research and development limited partnership).

Up till now, however the use of AFPs has not been applied on a commercial scale. Applicants are of the opinion that one of the reasons for the lack of commercial implementation is that although many AFPs have been described, in practice the implementation in actual commercial products encounters serious problems.

Applicants have found that one of the key reasons for these problems is that out of the great number of AFPs that have been described in the literature only a limited set of AFPs can suitably be applied for each application; also applicants have found that this selection of suitable AFPs is dependent on the desired application and/ or product attributes to be achieved.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques. The AFPs can suitably be used in food-products such as ice-cream. Example 3B shows modified ice-crystal shapes if a water-ice mixture is frozen into a film in combination with 0.01 wt% of AFP.

WO 92/22581 discloses AFPs from plants. This document also describes a process for extracting a polypeptide composition from extracellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

WO 94/03617 discloses the production of AFPs from yeast and their possible use in ice-cream. WO 96/11586 describes fish AFPs produced by microbes.

The present invention aims at providing frozen food products having a relatively hard and brittle texture, said texture being maintained upon prolonged storage at low temperatures.

A number of literature places have suggested that AFPs may potentially be used for favourably influencing the textural properties of frozen confectionery products such as ice

cream. However most of these documents do not provide a teaching how these favourable properties can actually be achieved in practice.

WO 96/11586 (not pre-published) teaches the application of fish antifreeze polypeptides in frozen fermented food products. Hard and brittle products are not mentioned.

WO 96/39878 (not pre-published) describes the application of AFP in ice-cream by using a specific freezing process. Suitable AFPs for this application may be derived from blood and muscle tissue of antartic fish, artic fish, worms and insects. Again hard and brittle products are not mentioned.

Surprisingly it has been found that AFPs can conveniently be incorporated in frozen food products to result in the desired product properties as long as the product and processing conditions are varied such that the ice-crystal shape satisfies specific requirements.

Accordingly in a first aspect, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of more than 1.9, preferably from 1.9 to 3.0.

If food products are frozen, ice-crystals are formed throughout the product. If AFPs are included in food products to be frozen this generally may lead to a favourable change in ice-recrystallisation properties. Aggregation of the ice-crystals of AFP containing products may cause the brittleness of the product.

Many consumers are in favour of relatively hard and brittle frozen food products or ingredients thereof such as ice-cream or water-ice. For example crispy water-ice can be used as an attractive ingredient in frozen confectionery products, also

relatively hard ice-cream is liked by a large group of consumers.

Surprisingly we have found that AFPs offer the opportunity to formulate frozen food products which on the one hand are relatively hard and brittle and on the other hand have improved ice-recrystallisation inhibition properties. Applicants have found that surprisingly this advantageous combination of properties can be achieved if the aspect ratio of the ice-crystals in the product is above 1.9, preferably between 1.9 and 3.

The aspect ratio of ice-crystals is defined as the ratio of the length and the breadth of the ice-crystals. An aspect ratio of above, preferably between 1.9 and 3 corresponds to elongated ice-crystals, which are not rounded in shape. The aspect ratio of crystals can be determined by any suitable method. A preferred method is illustrated in the examples. Preferably the ratio is between 2.0 and 2.9, most preferred between 2.1 and 2.8.

Preferably the frozen product of the invention are brittle. Preferably the minimum layer thickness at which fracture behaviour can be observed is less than 10 mm, more preferred from 1 to 5 mm. Fracture behaviour can either be measured by preparing layers of varying thickness and determining at which minimum thickness fracture behaviour occurs or calculated from the Young's Modulus as described in the examples.

During the formulation and subsequent freezing of food products several parameters can influence the aspect ratio of the ice-crystals to be formed. Examples of factors influencing the aspect ratio are given below. Applicants believe that it is well within the ability of the skilled person to choose those conditions such that the aspect ratio of the ice-crystals falls within the desired range.

One factor influencing the aspect ratio of ice-crystals is the rate of freezing the product. Generally speaking an increase of the rate of freezing leads to a decrease in the aspect ratio for the ice-crystals. In this context the temperature of freezing may influence the rate of freezing and therewith the aspect ration of the ice crystals. In this context freezing processes including a hardening step e.g. at a temperature below

-30 Fahrenheit are sometimes preferred. The storage temperature and storage time may equally influence the aspect ratio, whereby higher storage temperatures and/or longer storage times tend to favour the formation of high aspect ratios.

Another factor influencing the aspect ratio of ice-crystals is the mobility of the product during freezing. For example if a liquid water-ice or ice-cream mix is to be frozen, quiescently freezing will lead to a fairly high aspect ratio for the ice-crystals, while stirring leads to a lower aspect ratio. High shear mixing will lead to even lower aspect ratios.

Another factor to influence the aspect ratio of the ice crystals is the presence and amounts of ingredients. For example the presence of ingredients which tend to form a network structure in the product (e.g. gums or fats) may lead to a lower aspect ratio than the in products without these ingredients. Also other ingredients may lead to lower aspect ratios, for example high solids levels e.g. high sugar levels may lead to low aspect ratios.

Finally the nature and amount of the AFPs present may lead to a change in aspect ratios. Some AFPs seem to favour the formation of low aspect ratios, while other AFPs seem to induce higher aspect ratios. A suitable test to select these AFPs is described in the examples. Variation in the amount of AFPs may also lead to a change in aspect ratios.

According to a second embodiment, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the formulation, freezing and storage conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.

The process of the invention can be applied to any frozen food product containing AFPs. Examples of suitable products are sauces, meals etc. Preferred food products are frozen confectionery products such as ice-cream and water-ice.

Applicants have found that the AFPs for use in the process of the invention can come from a variety of sources such as plants, fishes, insects and microorganisms. Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.
Genetic manipulation techniques may be used to produce AFPs as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (eg in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish (i.e. they are either directly obtained from fish, or the fish proteins a transgenetically produced by other organisms). Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our non-prepublished case PCT/EP96/02936 (WO 97/2343).

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation -preferably measured in accordance to the examples- of less than 20 μm. more preferred from 5 to 15 μm. It is believed that the small ice-crystal size combined with the specific aspect ratio is especially advantageous to obtain the desirable structural features.

A very advantageous embodiment of the invention relates to product formulations which are chosen such that in the preparation of the product quiescent freezing conditions can be used, while still obtaining the aspect ratio as defined above.

Examples of such food products are: frozen confectionery mixes such as ice-cream mixes and water-ice mixes which are intended to be stored at ambient or refrigerator temperature. Suitable product forms are for example: a powder mix which is packed for example in a bag or in sachets. Said mix being capable of forming the basis of the frozen food product e.g.

after addition of water and optionally other ingredients and -optional- aeration.

Another example of a suitable mix could be a liquid mix (optionally aerated) which, if necessary after addition of further components and optional further aeration can be frozen.

The clear advantage of the above mentioned mixes is that the presence of the AFP ingredient enables the mixes to be frozen under quiescent conditions, for example in a shop or home freezer.

Very conveniently these mixes are packed in closed containers (e.g. cartons, bags, boxes, plastic containers etc). For single portions the pack size will generally be from 10 to 1000 g. For multiple portions pack sizes of up to 500 kg may be suitable. Generally the pack size will be from 10 g to 5000 g.

As indicated above the preferred products wherein the AFPs are used are frozen confectionery product such as ice-cream or water-ice. Preferably the level of AFPs is from 0.0001 to 0.5 wt% based on the final product. If dry-mixes or concentrates are used, the concentration may be higher in order to ensure that the level in the final frozen product is within the above ranges.

Surprisingly it has been found that compositions of the invention can contain very low amounts of AFPs while still being of good quality.

Up till now the general belief has been that fairly high levels of AFPs are required to obtain a reasonable improvement of recrystallisation properties. The reason for this is that it is commonly believed that the AFPs act on significant parts of the surface of the ice-crystals and

therefore need to be present at fairly high levels e.g. 0.01 wt% or more to get a reasonable effect.
Surprisingly it has now also been found that for frozen products improved recrystallisation properties and increased temperature tolerance can already be obtained if low levels of AFPs are used.

Surprisingly it has been found that the level of AFPs can be as low as 0.1 to 50 ppm while still providing adequate recrystallisation properties and temperature tolerance in frozen confectionery products. Although applicants do by no means wish to be bound by any theory, the reason for this may be that the interaction between the solids of the frozen confectionery and the AFPs provides an excellent mechanism for inhibiting crystal growth. Most conveniently the level of AFP is from 1 to 40 ppm, especially preferred from 2 to 10 ppm.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. For some applications the use of AFPs in frozen fermented food products is less preferred.

Preferably the level of solids in the frozen confection (e.g. sugar, fat, flavouring etc) is more than 4 wt%, prefably more than 30 wt%, more preferred from 40 to 70wt%.

In a very preferred embodiment of the invention the hard and crispy frozen confectionery formulations are used to create texture contrast in ice confections. Preferably such ice-confections contain as discrete elements in their structure the AFP containing composition in accordance to the invention. For example a relatively soft ice-cream core can be coated with a thin layer of the composition of the invention therewith providing a relatively hard and crispy

layer surrounding the ice-cream core. Another embodiment could be the incorporation of the formulation of the invention as inclusions in ice-confections. A third embodiment would be the alternating of layers of ice-cream with the formulation of the invention to create thin crispy layers alternating with the ice-cream layers.

Example I

A pre-mix for preparing ice-cream was made by mixing:

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
maltodextrine (MD40)	4.00
Locust bean gum	0.14
butteroil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (Type III HPLC-12, see WO 97/2343)	0.01 or none(control)
water	balance

This mix can conveniently be stored at ambient temperature e.g. in a plastic container.

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65°C followed by ageing over night at 5°C. The mix was frozen using a freezer (MF50 SSHE Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature was -4.5°C, the overrun was 110%. The product is then frozen at -35°C and stored at -80°C.

After two months storage the composition according to the invention had a markedly better texture than the control sample.

Example II

Pre-mixes for preparing ice-cream were made by mixing:

Formulation wt%	1	2	3	4	5	6
Butteroil	9.5	9.5	9.5	9.5	9.5	9.5
Milk Protein	18.5	18.5	18.5	18.5	18.5	18.5
Sucrose	12.2	12.2	10.2	10.2	7.4	7.4
Glucose Syrup	5.0	5.0	14.5	14.5	29.2	29.2
Mono glycerol palmitate	0.33	0.33	0.33	0.33	0.33	0.33
Guar gum	0.11	0.11	0.11	0.11	0.11	0.11
Locust Bean gum	0.11	0.11	0.11	0.11	0.11	0.11
Vanillin	0.01	0.01	0.01	0.01	0.01	0.01
AFP*	-	0.005	-	0.005	-	0.005
Water	to balance					
calculated fractional ice phase volume at -18°C	0.30	0.30	0.27	0.27	0.22	0.22

* Note AFP (Type III HPLC-12) as described in WO 97/02343

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65 °C followed by ageing over night at 5ºC. The mix was frozen using a freezer (MF50 SSHE Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature was -4.5°C, the overrun was 100%. The product is then frozen at -35°C and stored at -18°C.

Ice phase volumes were calculated by first calculating the amount of water that will freeze. This is done by the following method:

(a) For each ingredient, a plot is contructed showing freezing point depression against concentration of the ingredient in water;

(b) The amount of water bound by the ingredient at temperature T can then be calculated as:

$$Wi = (100/Ci - 1) * Si$$

whereby Ci is the concentration of the ingredient i that is required to depress the freezing temperature of water to the temperature T and Si is the concentration of the ingredient in the formulation.

(c) the calculation under (b) is repeated for each ingredient in the formulation and the total amount of water bound can then be calculated by adding up all the values Wi.

(d) the amount of water that will freeze can then be calculated by subtracting the total amount of water bound from the total amount of water in the formulation.

The rest of the calculation can then be done as follows (the formulas below are somewhat simplified, but sufficiently accurate to calculate the fractional ice phase volume of products of the invention):

1. Using the above method the amount of water (wt%) that will freeze at -18 °C can be calculated.

2. The volume fraction of the ice in the unaerated product can then be calculated as:

(p) (wt% of water that will freeze * density of total unaerated mix) / (density of ice * 100)

3. Similarly the initial volume fraction of the water that becomes ice can be calculated as:

(q) (wt% of water that will freeze * density of total mix)/ 100.

4. The total volume factor (wrt the unfrozen product) of the frozen unaerated product can then be calculated as:

(r) volume fraction of ice + (1 - volume of water that becomes ice) = (p) + (1 - (q))

5. The fractional ice phase volume of the unaerated product can be calculated by dividing the result of (p) by the result of (r):

(s) (p) / (r)

6. The fractional air phase volume can be calculated by:

(t) overrun %/ (100 + overrun%)

7. The fractional ice phase volume in the aerated product can then be calculated by:

(u) (1 - fractional air phase volume) * fractional ice phase volume of unaerated product = (1 - (t)) * (s)

Hardness measurements were made on the 6 formulations described above by using a Hounsfield HTE hardness tester at -18°C using a 10 mm diameter probe penetrating a block of the ice-cream at a rate of 400 mm/min to a depth of 20 mm. In these measurements an increased hardness corresponds to increased brittleness.

The following results were obtained:

Ice content @ -18°C (%)	Type III AFP level (%wt)	Hardness @ -18°C (N)
52	0	11.8 ± 3.0
	0.005	47.1 ± 12.1
45	0	7.2 ± 1.2
	0.005	25.3 ± 3.5
35	0	4.5 ± 1.1
	0.005	9.3 ± 2.6

This example clearly illustrates that if AFPs are added this leads to a clear increase of the brittleness of the product. By adding high levels of maltodextrin to the composition, a lower ice phase volume can be obtained. The examples show that if the fractional ice phase volume is below 0.27, surprisingly an acceptable brittleness can be obtained even in the presence of AFP.The above products 2, 4 and 6 were also subjected to sensory analysis. Products 2 and 4 scored significantly lower than product 6 on firmness, creamy texture and crumbliness. Again it was therefore confirmed that a lower ice phase volume leads to improved properties.

Example III

This example illustrates the method of calculation of the amount of water that will freeze for the following model formulation at -20°C :

Lactose	5.83
Sucrose	15.0
Water	79.17

(I) The amount of water prevented from freezing by sucrose

$$W_{sucrose} = ((100 / C_{sucrose}) - 1) * S_{sucrose}$$

From the plot of freezing point depression against solute concentration it can be seen that at -20°C, $C_{sucrose}$ = 70%

Therefore the amount of water prevented from freezing by sucrose is:

$$W_{sucrose} = ((100 / 70) - 1) * 15.0 = 6.43$$

(II) Similarly the amount of water prevented from freezing by lactose

$$W_{lactose} = ((100 / C_{lactose}) - 1) * S_{lactose}$$

From the plot of freezing point depresiion against solute concentration it can be seen that at -20°C, $C_{lactose}$ = 69%

Therefore the amount of water prevented from freezing =

$$W_{lactose} = ((100 / 69) - 1) * 5.83 = 2.62$$

The the total amount of water to be frozen (ice) is equal to:

$$W - W_{sucrose} - W_{lactose} = 79.17 - (6.43 + 2.62) = 70.12 \text{ wt\%}$$

Claims

1. A frozen food product comprising AFP, wherein the fractional ice phase volume in the product is below 0.27.

2. A frozen food product according to claim 1, wherein the fractional ice phase volume is from 0.05 to 0.26.

3. A frozen food product according to claim 1, wherein the ice phase volume is measured at -18 °C.

4. A frozen food product according to claim 1, wherein the ice phase colume is measured at a temperature between -2 and -15 °C.

5. A frozen food product according to claim 1, being a frozen confectionery product.

6. A frozen food product according to claim 1, wherein the level of AFP is from 0.0001 to 0.5 wt%.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/01577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23G9/02 A23L3/37

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23G A23C A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 234 611 A (RICH PRODUCTS CORP.) 18 November 1980 see column 4, line 60 - column 6, line 32; claim 1; example 1	1,2,4-6
Y	WO 97 02343 A (UNILEVER) 23 January 1997 see claims 1,5-7	1,2,4-6
A	EP 0 427 544 A (FUJI OIL) 15 May 1991 see column 1, line 1 - line 9; examples	1
A	WO 97 02754 A (UNILEVER) 30 January 1997 see page 1, line 22 - page 2, line 30; claim 1; example 1 see page 3, line 5 - line 6	1
A,P	WO 97 30599 A (UNILEVER) 28 August 1997	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
27 July 1998	04/08/1998

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International Application No
PCT/EP 98/01577

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 02567 A (AULT FOODS) 18 February 1993 see page 23, line 35 - page 24, line 4; claim 1 see page 26, line 37 - page 27, line 20 see page 1, line 15 - line 20 see page 2, line 6 - page 3, line 9 ---	1
A	EP 0 456 622 A (PERUCCI) 13 November 1991 ---	
P,A	WO 97 40700 A (UNILEVER) 6 November 1997 see examples ---	1
A	US 3 677 443 A (D C A FOOD INDUSTRIES) 18 July 1972 see column 1, line 6 - line 61 see column 3, line 15 - line 31 see column 5, line 53 - column 6, line 19 -----	1

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/01577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4234611 A	18-11-1980	US 4146652 A	27-03-1979
		US 4154863 A	15-05-1979
		US 4199604 A	22-04-1980
		US 4390550 A	28-06-1983
		US 4350711 A	21-09-1982
		AU 3281278 A	02-08-1979
		BE 863403 A	27-07-1978
		CA 1107120 A	18-08-1981
		DE 2803634 A	03-08-1978
		DE 2857879 C	08-01-1987
		DE 2858023 C	08-09-1988
		DE 2858058 C	24-01-1985
		DE 2858206 C	21-04-1988
		DE 2858207 C	08-10-1987
		DK 40078 A,B,	29-07-1978
		DK 504084 A,B,	22-10-1984
		FI 780232 A,B,	29-07-1978
		FI 831168 A,B,	06-04-1983
		FR 2378456 A	25-08-1978
		GB 1548358 A	11-07-1979
		JP 1187324 C	20-01-1984
		JP 53104767 A	12-09-1978
		JP 58020250 B	22-04-1983
		JP 1213082 C	27-06-1984
		JP 58047143 B	20-10-1983
		JP 58047450 A	19-03-1983
		NL 7801039 A,B,	01-08-1978
		NL 8303364 A,B,	01-02-1984
		NL 8303365 A	01-02-1984
		NL 8303366 A,B,	01-02-1984
		NL 8303367 A,B,	01-02-1984
		NL 8303368 A,B,	01-02-1984
		SE 439577 B	24-06-1985
		SE 7800962 A	29-07-1978
		US 4244976 A	13-01-1981
		US 4244977 A	13-01-1981
		US 4308287 A	29-12-1981
		US 4237146 A	02-12-1980
		US 4199605 A	22-04-1980

Form PCT/ISA/210 (patent family annex) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/01577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9702343 A	23-01-1997	AU 6519096 A	05-02-1997
		EP 0836646 A	22-04-1998
EP 427544 A	15-05-1991	JP 1961019 C	10-08-1995
		JP 4104765 A	07-04-1992
		JP 6087745 B	09-11-1994
		JP 3151831 A	28-06-1991
		JP 6087744 B	09-11-1994
		DE 69005417 D	03-02-1994
		DE 69005417 T	07-04-1994
		US 5120566 A	09-06-1992
WO 9702754 A	30-01-1997	AU 6301196 A	10-02-1997
		EP 0837635 A	29-04-1998
WO 9730599 A	28-08-1997	AU 1768197 A	10-09-1997
WO 9302567 A	18-02-1993	US 5215777 A	01-06-1993
		AU 2394792 A	02-03-1993
		CA 2050429 A	17-11-1992
		MX 9204929 A	01-02-1993
EP 456622 A	13-11-1991	IT 90047926 A	08-11-1991
WO 9740700 A	06-11-1997	AU 2386797 A	19-11-1997
US 3677443 A	18-07-1972	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)